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# ATORVASTATIN, AN HMG-COA REDUCTASE INHIBITOR AND EFFECTIVE LIPID-REGULATING AGENT. PART II.[1] SYNTHESIS OF SIDE-CHAIN-LABELED [$^{14}$C]ATORVASTATIN.

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## SUMMARY

[$^{14}$C]Atorvastatin was synthesized in a ten-step sequence with an overall yield of 5.7%. The label was introduced as sodium [1-$^{14}$C]acetate, which was converted via the acid chloride to the (*S*)-2-hydroxy-1,2,2-triphenylethyl ester **4**. Chiral condensation of **4** with aldehyde **5** gave the chiral ester intermediate **6** with a yield of about 70%. Following transesterification of **6** to a methyl ester and condensation with *tert*-butyl lithioacetate to give (*R*)-ß-ketoester **8**, a second chiral center was generated by reduction of the hydroxyketone **8**, giving the (*R,R*)-dihydroxy ester **9**, which was then converted via the acid to the lactone **11**. The desired pure diastereomer **11**, obtained from the mother liquor during crystallization, was then converted to the corresponding calcium salt (2:1) **13** (atorvastatin).

**Keywords:** HMG-CoA reductase inhibitor, atherosclerosis, $^{14}$C side-chain labeled atorvastatin, Lipitor®, hypocholesterolemia agent.

## INTRODUCTION

It is well established that inhibition of the rate-limiting enzyme in the biosynthetic pathway of cholesterol, HMG-CoA reductase (HMGR), is an effective means for lowering plasma cholesterol level in hypercholesterolemic individuals. Agents such as lovastatin, simvastatin, and pravastatin have been highly effective in lowering both total and LDL cholesterol. Atorvastatin (Lipitor®), the newest member of this class of inhibitors, has demonstrated enhanced effectiveness[2] as well as other unique properties such as reduction of triglycerides and apo B and elevation of HDL cholesterol.[3,4] During its development, two C-14 labeled forms of atorvastatin were synthesized for pharmacokinetics and metabolism studies.

This paper describes the first synthesis, where the label was on the side chain. Subsequently, because of concern over the possibility of the N-dealkylative loss of the radioactive side-chain, which might then enter other endogenous metabolic pathways, a second labeled form where the label resides in the central pyrrole ring was synthesized, as indicated in Part 1 of the series.[1] However, the actual occurrence of N-dealkylation has not been substantiated in subsequent metabolic studies.[5]

## RESULT AND DISCUSSION

The synthesis of the side-chain-labeled atorvastatin, an adaptation of the methodology of Roth, et al.,[6] is shown in Scheme 1. The label was introduced with sodium [1-$^{14}$C]acetate (**1**) to give

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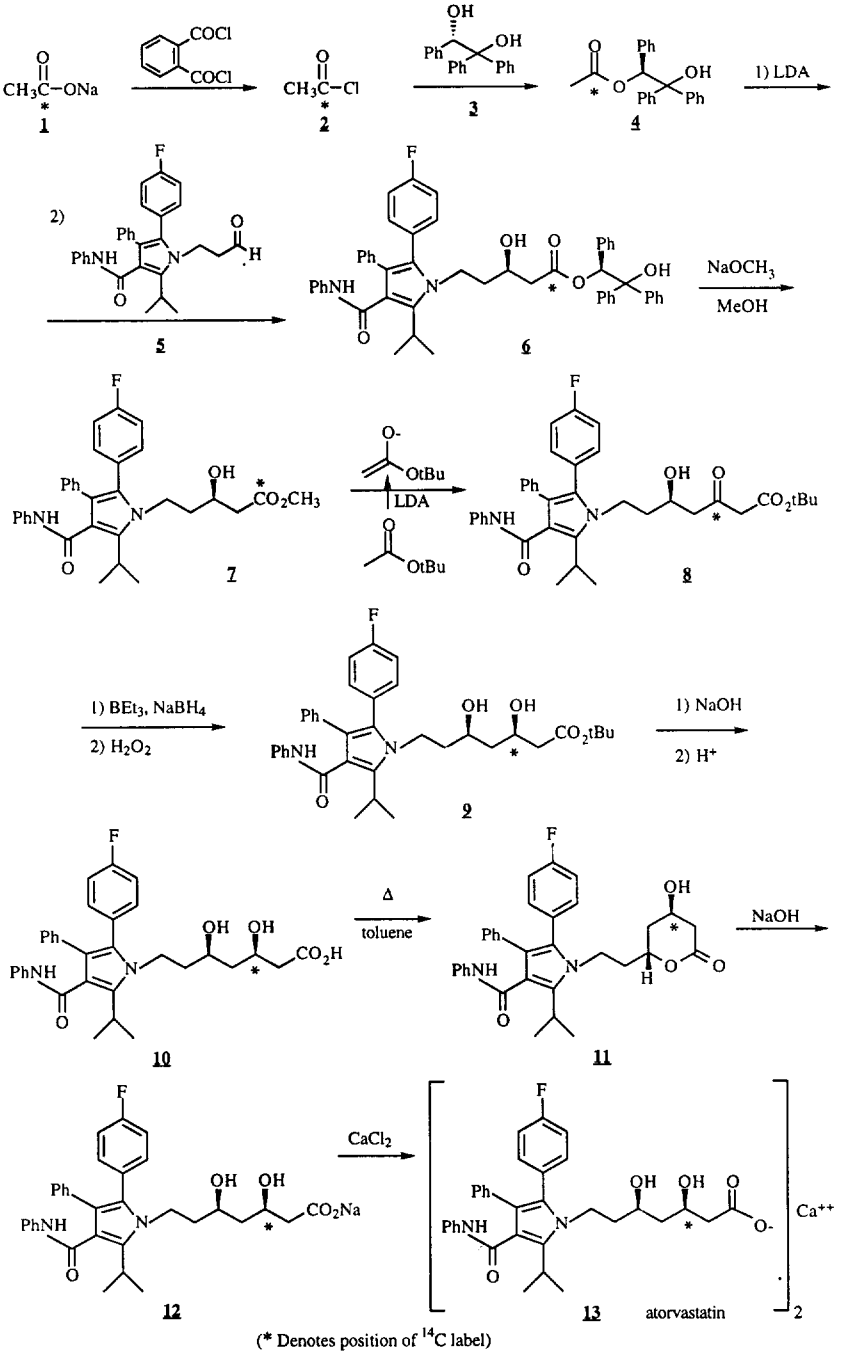
**Scheme I. Synthetic Scheme for Side-Chain-Labeled [$^{14}$C]CI-981**



[1-$^{14}$C]acetyl chloride **2**, which was then esterified with chiral diol **3**[7] to give ester **4**. The presence of the chiral auxiliary enabled the chiral condensation with aldehyde **5**[6] to give **6** with a yield of about 70%. Following transesterification of **6** to a methyl ester, condensation with *tert*-butyl lithioacetate gave the (*R*)-ß-ketoester **8**. A second chiral center was generated by chiral reduction of **8** with triethyl borane and sodium borohydride, giving the *R,R*-dihydroxy ester **9**, which was then converted via the acid to the lactone **11**. Chromatographic purification of the lactone, followed by recrystallization, removed any remaining undesired stereoisomers as the crystalline fraction, and the desired pure diastereomer **11** was obtained from the mother liquor. The lactone was then converted to the calcium salt (2:1) **13**. Side-chain labeled [$^{14}$C]atorvastatin was thus synthesized in a ten-step sequence with an overall yield of 5.7%.

## EXPERIMENTAL

**General.** Radioactivity was determined with a Packard Tri-Carb 4530 liquid scintillation counter, using Beckman Ready-Gel as the counting medium. TLC was performed with Silica Gel 60 $F_{254}$ precoated plates by EM Science and were scanned on a Berthold LB2832 automatic TLC linear analyzer. HPLC was performed with Alltech Econosil C18-10µ analytical columns, 4.6 mm x 250 mm, unless otherwise specified. THF (tetrahydrofuran) was purified by distillation over sodium and benzophenone. Drying of non-hydroxylic solvents, if indicated, was generally accomplished with Molecular Sieves. All labeled compounds synthesized (except [1-$^{14}$C]acetyl chloride) were identified by TLC or HPLC comparison, or both, with the corresponding authentic unlabeled compounds.

**[1-$^{14}$C]Acetyl chloride (2).** Prior to use, the commercially purchased [1-$^{14}$C]sodium acetate was dried in high vacuum under refluxing xylene for 24 h. Phthaloyl chloride (9.62 g, 47.38 mmol) was added by injection to the dried sodium [1-$^{14}$C]acetate (0.778 g, 9.476 mmol) under an inert atmosphere, and the mixture was heated at 150 °C for 3 h. The resulting [1-$^{14}$C]acetyl chloride was collected by vacuum transfer (quantitative).

**[1-$^{14}$C]Acetic acid, (*S*)-2-hydroxy-1,2,2-triphenylethyl ester (4).** [1-$^{14}$C]Acetyl chloride (0.887 g, 11.22 mmol) was added to a solution of compound **3** (3.26 g, 11.22 mmol) in 25 mL $CH_2Cl_2$, and the mixture was stirred at room temperature for 2 h. The white precipitate was filtered, washed with $CH_2Cl_2$, then with water. The product was collected and dried under vacuum for 24 h (1.1 g, 30%).

**(*R*)-5-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrol-1-yl]-3-hydroxy-[1-$^{14}$C]pentanoic acid, (*S*)-2-hydroxy-1,2,2-triphenylethyl ester (6).** LDA (lithium aluminum hydride) (15.1 mmol in 30 ml THF) was added to a suspension of **4** (2.51 g, 7.55 mmol) in 20 ml of THF at -78 °C, and the mixture was slowly warmed to -10 °C during 1.5 h. Aldehyde **5** (3.6 g, 7.92 mmol) was added to the mixture after it was cooled to -78 °C, and the reaction was kept at this temperature for 2 h. The solution was quenched with 5 mL of glacial acetic acid, and the mixture was allowed to warm up to room temperature. The solvent was evaporated, and the solid was washed with water, filtered, and dried under vacuum (4.00 g, 67.8%).

**(*R*)-5-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrol-1-yl]-3-hydroxy-[1-$^{14}$C]pentanoic acid methyl ester (7).** Sodium methoxide (0.43g, 8 mmol) was added to a yellow solution of **6** (4.00 g, 5 mmol) in 30 mL of dry THF and 17.5 mL of dry methanol at 0 °C in one portion. The mixture was stirred at 0 to 5 °C for 3 h, treated with HOAc (0.5 mL), and concentrated to a yellow solid. The solid was partitioned between EtOAc and water. The organic layer was separated from the aqueous portion, and the aqueous portion was extracted with EtOAc (3x20 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and evaporated to dryness to give 1.4 g (52%) of product.

**(*R*)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrol-1-yl]-5-hydroxy-3-oxo-[1-$^{14}$C]heptanoic acid *tert*-butyl ester (8).** *tert*-Butyl acetate (3.57 mL, 26.5 mmol) was added to a solution of LDA (26.5 mmol) in 40 mL of hexane at -78 °C and stirred at this temperature for 1 h. Compound **7** (1.4 g, 2.65 mmol) in 5 mL of THF was added slowly to the mixture, and the temperature was slowly raised to -40 °C for 3 h, and then warmed up to -10 °C for 2 h. Conc. HCl (5 mL) was added and the reaction was concentrated to dryness. The residue was

distributed between EtOAc and $H_2O$. The organic layer was washed with brine, $NH_4Cl$, saturated $NaHCO_3$. After column chromatography, the purified product weighed 1.25 g (77%).

**[*R*-(*R**,*R**)]-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-pyrrol-1-yl]-3,5-dihydroxy-[1-$^{14}$C]heptanoic acid tert-butyl ester (9).** ß-Ketoester **8** (1.25 g, 2.04 mmol) in 5 mL THF was added to a mixture of 2,2,2-trimethylacetic acid (0.104 g, 0.102 mmol) and $Et_3B$ (0.201g, 2.05 mmol) under $N_2$, and stirred at room temperature for 1 h, before cooling to -78 °C. MeOH (2.76 mL) was added at this temperature, followed by $NaBH_4$ (0.096 g, 2.55 mmol) in three portions at 5-min. intervals, and the temperature was kept at -70 °C during the addition. Stirring was continued for another 2.5 h after the addition; 30% $H_2O_2$ (5.8 mL) and $H_2O$ (10 mL) were then added at this temperature, and the reaction mixture was allowed to warm to room temperature slowly. $CHCl_3$ (20 mL) and $H_2O$ (20 mL) were added to the mixture, and the water layer was extracted with $CHCl_3$ (3x10mL). The organic layers were combined and washed with water until no peroxide was present. The organic portion was dried and evaporated to dryness. After purification by column chromatography (EtOAc:hexane/1:1), compound **9** was obtained as white powder (1.1 g, 88%).

**[*R*-(*R**,*R**)]-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-pyrrol-1-yl]-3,5-dihydroxy-[1-$^{14}$C]heptanoic acid (10) and δ-lactone (11).** Dihydroxy ester **9** (1.1 g, 1.8 mmol) was dissolved in 10 mL of THF, and 2 mL 3 N NaOH was added with stirring at room temperature. After 4 h the solvent was removed, and the residue was dissolved in 10 mL of $H_2O$. The aqueous solution was extracted with ether (2x10 mL) and then acidified with 1 N HCl. Acid **10** was extracted with EtOAc (3x20 mL), and the extracts were dried over $MgSO_4$ and evaporated to dryness. The residue was dissolved in 50 mL of toluene and heated under reflux for 3 h, with removal of water by a Dean-Stark trap. The solvent was evaporated, and the resulting lactone **11** was isolated by column chromatography (EtOAc:hexane/4:1). Appropriate lactone fractions were combined and evaporated, and the residue was recrystallized twice from EtOAc/hexane. Any undesired stereoisomers were removed as crystals, leaving the pure (*R,R*)-lactone **11** in the mother liquor, as evidenced by HPLC with a Chiralcel OF column (hexane:EtOAc/80:20; 1 mL/min, 254 nm) (Diacel Chem. Ind., Ltd.). The weight of optically pure **11** was 0.32 g (34% yield). The chemical and radiochemical purities were both >99%. TLC, silica gel, EtOAc:hexane/ 4:1, Rf 0.36. HPLC, Alltech Econosil C-18, 10µ, 0.05M citric acid (adjusted to pH 4.0 with $NH_4OH$): $CH_3CN$/1:1, flow rate 2 mL/min, retention time 6.5 min, UV detection at 254 nm).

**[*R*-(*R**,*R**)]-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-pyrrol-1-yl]-3,5-dihydroxy-[1-$^{14}$C]heptanoic acid calcium salt (2:1) (13),** i.e., [*R*-(*R**,*R**)]-2-(4-fluorophenyl)-ß,γ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)car-bonyl]-1H-pyrrole-1-[1-$^{14}$C]heptanoic acid calcium salt (2:1). Sodium hydroxide (1 N, 0.351 mL) was added to a solution of **11** (190 mg, 0.351 mmol) in THF (3 mL) and MeOH (1 mL), and the mixture was stirred at room temperature for 2 h. TLC (EtOAc:hexane/4:1) showed that **11** had completely disappeared. $CaCl_2$ solution (19.52 mg, 0.355 meq., 0.5 mL) was then added, and stirring was continued for one more hour. The solvent was evaporated, and the white residue was washed with water and dried to give the calcium salt **13**, 78.6µCi/mg. HPLC (same system as for **10** and **11** above): radiochemical purity 98.71%; chemical purity (254 nm), 99.61%.[8]

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8. Besides HPLC, the labeled compound **13** was characterized by NMR and IR comparisons with an authentic reference standard. Chemical purity by HPLC is defined as purity by area normalization at a chosen wavelength (e.g., 254 nm for **13**, as specified for purity determination of the drug), generally near the absorption maximum. As a calcium salt, atorvastatin typically melts with decomposition over a wide temperature range of about 160 to 240 °C.[1]